

Research article

Enhancing Marigold Seed Quality During Storage Through Fungicide Pelleting and Suppression of *Pythium* sp. at the Seedling Stage

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Abstract

Marigold seeds are small, lightweight, and have limited nutrient reserves, leading to issues with slow and uneven germination. Seed pelleting technology has been utilized to increase the size and uniformity of seeds. Additionally, marigolds are susceptible to *Pythium* sp., a fungal pathogen causing damping-off disease, characterized by water-soaked lesions at the stem base, leading to seedling collapse and death. This study aimed to identify the appropriate type and concentration of fungicidal agents for pelleting marigold seeds to prevent *Pythium* sp. infection while maintaining seed quality both before and after storage. The experiments were designed using a completely randomized design (CRD) with four replications. The results indicated that mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. were the optimal types and concentrations for seed pelleting. Specifically, pelleting with mancozeb at 0.4 g.ai. significantly enhanced seed germination, vigor, and seedling growth compared to other methods. Furthermore, seeds pelleted with mancozeb at 0.4 g.ai. maintained high germination rates after two months of storage under both controlled and ambient conditions when tested in laboratory settings. In greenhouse conditions, seeds pelleted with mancozeb at 0.4 g.ai. showed high seedling viability two months post-storage under both controlled and ambient conditions. Therefore, pelleting marigold seeds with mancozeb at 0.4 g.ai. is recommended as it promotes germination, enhances seedling vigor and growth, and effectively inhibits *Pythium* sp. throughout storage under both conditions.

Keywords: seed enhancement; seedling disease; damping-off disease; seed rot

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1. Introduction

Pythium sp. are fungus-like organisms responsible for damping-off disease in the seedlings of many economically important plants. Classified as water molds, *Pythium* spp. produce coenocytic hyphae and reproduce asexually through the formation of zoospores within vesicles, and sexually through the formation of oospores (Calvet et al., 1993; Ali et al., 2019). These soil-borne pathogens can survive as saprophytes, infecting nearly all plant species indiscriminately, making them a significant cause of seedling damping-off. This disease manifests as seed rot, root rot, and collar rot by attacking delicate tissues, affecting seedlings both pre- and post-emergence (Arora et al., 2021). The rapid spread of this disease depends on the fungal species and environmental conditions.

Marigold (*Tagetes erecta* L.), belonging to the Asteraceae family, is a widely cultivated ornamental flower of great economic importance in Thailand. During cultivation, seedlings are typically raised in nursery trays before being transplanted. The growing media often contain various components that may harbor *Pythium* sp. Marigold seeds, being small, flat, elongated, and lightweight, pose challenges in handling and sowing (Kangsopa et al., 2024). They also have limited food reserves, leading to uneven seedling growth and weak plants, making them vulnerable to *Pythium* sp. This results in pre-emergence damping-off, characterized by seed rot, softening, browning, and eventual decay (Calvet et al., 1993). In newly germinated seedlings, the radicle, hypocotyls, and plumule may exhibit water-soaked lesions, leading to rapid seedling death and failure to emerge above the soil surface. Post-emergence damping-off is observed as seedling collapse, with water-soaked lesions at the soil line, progressing to brown necrotic lesions that cause the seedlings to topple and die (Hyder et al., 2018; Dubey et al., 2020; Arora et al., 2021).

Given these challenges, marigold seeds face significant pre- and post-emergence issues, causing economic losses to growers. Seed pelleting technology, which involves coating seeds with materials to increase their size and change their shape (Taylor et al., 1998), can enhance the handling and sowing efficiency of small marigold seeds. Pelleting also protects seeds during sowing and storage by reducing mechanical damage and minimizing the risk of fungal infection. The incorporation of fungicides such as metalaxyl, mancozeb, imidacloprid, and fosetyl-aluminium chemicals known to inhibit the mycelial growth of *Pythium* sp. (Chatchaisiri et al., 2018) into the pelleting process can enhance seed germination and seedling development by preventing infections caused by *Pythium* sp. Kangsopa (2015) reported that pelleting tobacco seeds with captan at 4 and 6 g.ai., metalaxyl at 2, 4, and 6 g.ai., and copper hydroxide at 4 and 6 g.ai., effectively inhibited *Pythium* sp. by 100%, whereas untreated seeds showed over a 50% increase in disease incidence.

The appropriate type and concentration of fungicides is crucial for effectively managing marigold diseases. This study aimed to isolate *Pythium* sp. responsible for damping-off disease in marigold and to evaluate the in vitro efficacy of six fungicides against the pathogen. The effects of seed pelleting with varying concentrations of mancozeb and fosetyl-aluminium on seed germination, seedling growth, and disease suppression were also examined. Furthermore, the study assessed the influence of these treatments on seed quality and storage performance under both controlled and ambient conditions to determine the most effective fungicide type and concentration for maintaining marigold seed viability and health.

2. Materials and Methods

2.1 Location of experiment, duration, and seed quality

The experiment was conducted at the Seed Technology Laboratory, Plant Disease Laboratory, and the Modern Seed Technology Research Center of the Agronomy Program, Faculty of Agricultural Production, Maejo University, Chiang Mai, Thailand. The study was carried out from March 2023 to January 2024. The initial germination percentage of the marigold seeds used in the experiment was 49%.

2.2 Preparation of *Pythium* sp.

Marigold plants exhibiting symptoms consistent with *Pythium* sp. infection were collected from agricultural fields in San Sai District, Chiang Mai Province. The affected plants displayed water-soaked, darkened, and decayed tissues at the stem base. Prominent dark brown lesions were observed on the stems, and in severe cases, stem breakage occurred. The collected samples were thoroughly washed with clean water to remove any adhering soil. Subsequently, the samples were cut into small pieces of approximately 1-2 cm in length and subjected to surface sterilization. The sterilization process comprised three steps: 1) immersing in 70% ethanol (v/v) for 1 min, 2) soaking in a 2% sodium hypochlorite solution (v/v) for 2 min, and 3) rinsing with autoclaved distilled water. Following sterilization, the plant pieces were placed on sterile marigold medium under aseptic conditions and incubated at room temperature for 7 days. After the incubation period, hyphae of *Pythium* sp. were transferred to Potato Dextrose Agar (PDA) to obtain pure cultures. These cultures were maintained at room temperature ($25\pm 2^\circ\text{C}$) for 3 days to allow for growth and establishment (Chuenchan et al., 2019).

2.3 Evaluation of the inhibition of *Pythium* sp.

The antifungal activity of six commercially available fungicides with known efficacy against *Pythium* sp. was evaluated at the concentrations recommended on their respective product labels. The fungicides tested included metalaxyl (25% WP), captan (50% WP), mancozeb (80% WP), imidacloprid (70% WG), fosetyl-aluminium (80% WG), and chlorothalonil (75% WP). The poisoned food technique, as described by Adjou et al. (2012), was employed to assess their inhibitory effects. Specific concentrations of each fungicide were prepared and incorporated into cooled molten PDA (45°C). The fungicide solutions were manually mixed in a sterile Erlenmeyer flask to ensure even dispersion. Twenty milliliters of the medium was dispensed into sterile petri dishes (9 cm in diameter), with care taken to avoid trapping air bubbles. The medium was allowed to solidify at room temperature ($25\pm 2^\circ\text{C}$) for approximately 1 h. Agar discs with mycelia (6 mm in diameter) were cut from the periphery of actively growing regions of 3-day-old pure cultures using a sterile cork borer and aseptically inoculated at the center of the petri plates. Control plates, without fungicides, were prepared following the same procedure. Each treatment was conducted in triplicate, and the plates were incubated at $25\pm 2^\circ\text{C}$. Fungal colony diameters were measured after one and three days of incubation. The percentage inhibition of mycelial growth of *Pythium* sp. by each fungicide was calculated using the formula provided by Philippe et al. (2012).

2.4 Marigold seed pelleting with fungicides

A 0.3% w/w aqueous solution of carboxymethyl cellulose (CMC, Sigma Aldrich) was formulated to act as the binding agent for the seed pelleting process. Vermiculite (Union Chemical Ltd., Bangkok, Thailand) was used as a pelleting material at a quantity of 100 g of marigold seed. Using a pipette, filler materials were carefully applied to the seeds with the CMC solution, resulting in the following treatments: non-pelleted seeds (control), T2 = pelleted seeds with vermiculite, T3 = pelleted seeds with mancozeb at 0.1 g.ai./100 g of seed, T4 = pelleted seeds with mancozeb at 0.2 g.ai./100 g of seed, T5 = pelleted seeds with mancozeb at 0.3 g.ai./100 g of seed, T6 = pelleted seeds with mancozeb at 0.4 g.ai./100 g of seed, T7 = pelleted seeds with fosetyl-aluminium at 0.1 g.ai./100g of seed, T8 = pelleted seeds with fosetyl-aluminium at 0.2 g.ai./100 g of seed, T9 = pelleted seeds with fosetyl-aluminium at 0.3 g.ai./100 g of seed, and T10 = pelleted seeds with fosetyl-aluminium at 0.4 g.ai./100 g of seed. The pelleting procedure was conducted using a Model SKK12 rotary drum (Seeds Processing Plant, Khon Kaen University, Thailand) rotating at 42 rpm. After pelleting, the seeds were air-dried at room temperature until their moisture content reached 7.5%.

2.5 Seed storage

All seed treatments were packed in aluminum foil bags (10×15 cm, W×L) and then stored under either ambient condition (27°C±2 and 80%±5 relative humidity) or controlled conditions (4°C and 50% relative humidity). Seed quality was assessed every two months over a six-month period.

2.6 Seed testing under laboratory and greenhouse conditions

For seed testing under laboratory conditions, 50 marigold seeds were tested using transparent plastic boxes (110 × 110 × 30 mm, length × width × height) and the Top of Paper (TP) method, with four repetitions. The seeds were placed in a germination incubator and maintained at 25°C and 80% relative humidity, with continuous light exposure at 180 µE for 24 h. The germination percentage was assessed by counting the number of normal seedlings on days 5 (first count) and 14 (final count) (ISTA, 2023). The speed of germination was monitored daily by recording the number of normal seedlings from days 5 to 14 (AOSA, 1983). The mean germination time (MGT) was calculated using the formula: $MGT = \sum(n \times d) / N$, where n represents the number of seeds germinated each day, d is the number of days since the test began, and N is the total number of germinated seeds at the end of the experiment (Ellis & Roberts, 1981). On day 14 after sowing, ten seedlings were randomly selected to measure mean shoot length, root length, shoot fresh weight, and shoot dry weight (Jeepet et al., 2023). Under greenhouse conditions, the germination testing of marigold seeds was conducted using seed trays filled with peat moss (Klasmann-Deilmann GmbH, Ltd., Germany) as the seeding medium. Germination percentage, speed of germination, and mean germination time were assessed following the same procedures used under laboratory conditions. On day 14 after sowing, ten seedlings were randomly selected to measure the mean shoot length and shoot fresh weight (Jeepet et al., 2023).

2.7 Statistical analysis

The percentage of germination was arcsine transformed into normalization the data before statistical analysis. All data were analyzed by one-way analysis of variance (ANOVA, Complete Randomized Design), and the differences between the treatments were tested at the significant level of $P \leq 0.05$ by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Inhibition of *Pythium* sp. by fungicides

Before applying fungicides to marigold seeds, it is crucial to identify the type of fungicides that can effectively inhibit *Pythium* sp. In this study, six different fungicides were tested for their efficacy against *Pythium* sp. The results showed that mancozeb and fosetyl-aluminium could inhibit *Pythium* sp. by 100%, while metalaxyl, captan and chlorothalonil achieved 76%, 60% and 24% inhibition, respectively. Imidacloprid, however, was unable to inhibit *Pythium* sp. (Table 1). Mancozeb, a member of the dithiocarbamate group, functions as a contact fungicide by inhibiting spore formation, spore germination, and mycelial development. Its mechanism of action involves mancozeb's dialkyl dithiocarbamates, which chelate heavy metals and interact with proteins and enzymes, causing them to malfunction. Additionally, mancozeb releases free radicals that interfere with organic compounds in the cells, leading to cellular dysfunction in the pathogen. Monoalkyl dithiocarbamates also release toxic isothiocyanates that bind to the sulphhydryl groups in fungal cellulose-based cell walls, effectively halting hyphal growth (Elsharkawy et al., 2019; Kumar et al., 2023). Fosetyl-aluminium, a phosphonate, is a systemic fungicide that inhibits spore germination and disrupts hyphal development. Its mode of action involves competing with or displacing phosphates in the function of allosteric regulators in various enzymes (Gormez et al., 2021). In contrast, imidacloprid was ineffective against *Pythium* sp. As shown in Figure 1, no mycelium of *Pythium* sp. was observed growing on the culture medium containing mancozeb (Figure 1D) and fosetyl-aluminium (Figure 1F). Chlorothalonil showed only 24% inhibition of *Pythium* sp., possibly due to its limited systemic activity and lack of specificity toward oomycete targets. As a non-systemic, multi-site contact fungicide, chlorothalonil acts primarily by disrupting thiol-containing enzymes in fungal spores and mycelia, but it is less effective against oomycetes, which have distinct biochemical pathways and sterol compositions (Martin et al., 2003). Imidacloprid is a heterocyclic aromatic organic compound with two nitrogen atoms in its ring structure, making it a weak base. This characteristic may reduce its efficacy under unsuitable environmental conditions, such as inappropriate pH levels that accelerate chemical hydrolysis. Furthermore, the compound is susceptible to rapid photodegradation when exposed to high-intensity sunlight. Additionally, Imidacloprid typically inhibits ergosterol synthesis in fungal cells, which is a critical component of fungal cell membranes. However, *Pythium* sp. may have cell membrane structures that do not rely as heavily on ergosterol compared to other fungi, rendering imidacloprid less effective against them (Ayesha et al., 2021; Gormez et al., 2021).

Table 1. Inhibition of *Pythium* sp. mycelial growth by different fungicides using the poisoned food technique after incubation at 25±2°C

Treatment	Inhibition of <i>Pythium</i> sp. ¹ (%)
Metalaxyl (25% WP)	76 ^{ab}
Captan (50% WP)	60 ^b
Mancozeb (80% WP)	100 ^a
Imidacloprid (70% WG)	0 ^c
Fosetyl-aluminium (80% WG)	100 ^a
Chlorothalonil (75% WP)	24 ^{bc}
F-test	**
C.V.%	6.44

** : significant difference at $P \leq 0.01$.

¹Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

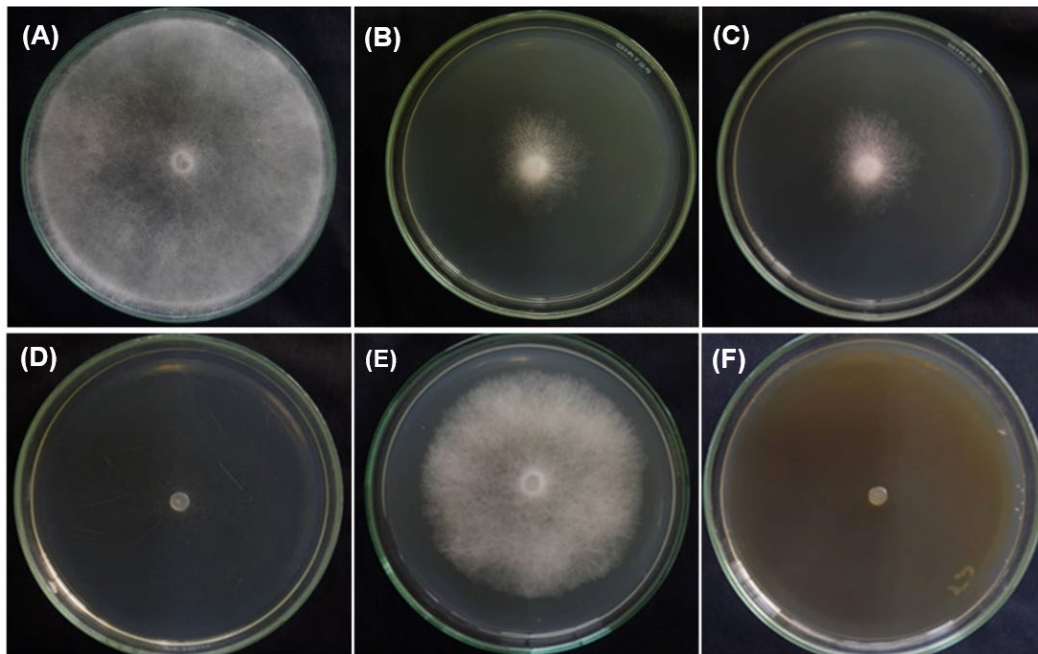


Figure 1. Characteristics of growth inhibition of *Pythium* sp. fungal hyphae by different fungicides using the poison food technique. (A) Chlorothalonil (75% WP), (B) Captan (50% WP), (C) Metalaxyl (25% WP), (D) Fosetyl-aluminium (80% WG), (E) Imidacloprid (70% WG), and (F) Mancozeb (80% WP).

3.2 Effectiveness of fungicides on *Pythium* sp. inhibition and seed quality

After selecting two fungicides, mancozeb and fosetyl-aluminium, for their efficacy in inhibiting *Pythium* sp., further tests were conducted to evaluate their inhibitory effects at four different concentration levels. The experimental results demonstrated that mancozeb at concentrations of 0.3 g.ai. and 0.4 g.ai., and fosetyl-aluminium at concentrations of 0.2 g.ai., 0.3 g.ai., and 0.4 g.ai., could inhibit *Pythium* sp. by 100%. In contrast, mancozeb at 0.1 g.ai. and 0.2 g.ai., and fosetyl-aluminium at 0.1 g.ai., inhibited *Pythium* sp. by 86%, 84%, and 87%, respectively (Table 2). These findings indicate that mancozeb must be used at concentrations of 0.3 g.ai. or higher to achieve 100% inhibition of the fungus. The efficacy of mancozeb can be attributed to its composition, which includes heavy metals that interfere with the proteins and enzymes in the mycelium of *Pythium* sp., effectively halting their growth. At concentrations of 0.3 g.ai. and above, the monoalkyl dithiocarbamates in mancozeb release toxic isothiocyanates that bind to the sulphhydryl groups in the cellulose-based cell walls, significantly impacting the growth of *Pythium* sp. (Elsharkawy et al., 2019; Kumar et al., 2023). For fosetyl-aluminium, the results indicated that a concentration of 0.2 g.ai. or higher is required to achieve 100% inhibition of *Pythium* sp. Fosetyl-aluminium, a systemic fungicide, acts by competing with or displacing phosphates in the function of allosteric enzymes, which are multi-subunit enzymes that regulate fungal enzyme activity (Ayesha et al., 2021; Gormez et al., 2021). At concentrations below 0.2 g.ai., the inhibitory effect is not fully effective, but at concentrations above 0.2 g.ai., fosetyl-aluminium effectively inhibits the growth of *Pythium* sp. by 100%.

Table 2. Inhibition of *Pythium* sp. mycelial growth by different concentrations of mancozeb and fosetyl-aluminum using the poisoned food technique after incubation at 25±2°C

Treatment	Inhibition of <i>Pythium</i> sp. ¹ (%)
Mancozeb 0.1 g.ai.	86 ^b
Mancozeb 0.2 g.ai.	84 ^b
Mancozeb 0.3 g.ai.	100 ^a
Mancozeb 0.4 g.ai.	100 ^a
Fosetyl-aluminium 0.1 g.ai.	87 ^b
Fosetyl-aluminium 0.2 g.ai.	100 ^a
Fosetyl-aluminium 0.3 g.ai.	100 ^a
Fosetyl-aluminium 0.4 g.ai.	100 ^a
F-test	**
C.V.%	5.4

** : significantly different at $P \leq 0.01$.

¹Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

After selecting two effective fungicides, mancozeb and fosetyl-aluminium, the fungicides were further tested by dividing them into four different concentration levels and applying them to marigold seeds to evaluate their impact on seed quality. Under laboratory conditions, the results revealed that seed pelleting with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. resulted in significantly higher germination rates compared to non-pelleted seeds. Additionally, mancozeb at 0.4 g.ai. exhibited a faster germination rate than

other treatments. However, all seed pelleting treatments (T2-T10) showed a slightly slower mean germination time compared to the non-pelleted seeds. Under greenhouse conditions, seeds pelleted with mancozeb at 0.4 g.ai. continued to exhibit higher germination rates and speed of germination than other treatments. Non-pelleted seeds and only pelleted seeds showed slightly better mean germination times compared to all fungicide-treated seeds (T3-T10). Considering the seed quality after pelleting with the two fungicides, it was found that pelleting with mancozeb at 0.4 g.ai. did not negatively impact seed germination and vigor (Table 3). Mancozeb, a contact fungicide, inhibits spore formation, spore germination, and fungal mycelium development. It has a broad-spectrum activity due to its composition, including zinc and manganese. At concentrations lower than 0.4 g.ai., seed germination rates did not differ significantly, but at 0.4 g.ai., the germination rate increased. This could be attributed to zinc, a key component in mancozeb, enhancing the activity of enzymes involved in germination, particularly those related to protein synthesis and nucleic acid synthesis, which are crucial for cell division during germination. Zinc also strengthens the plant's immune system, helping it resist diseases and adverse environmental conditions (Stanković et al., 2010). Reports indicate that pelleting corn seeds with mancozeb at 2, 4,

Table 3. Germination percentage (GE), speed of germination (SGE), and mean germination time (MGT) of marigold seeds pelleted with different concentrations of mancozeb and fosetyl-aluminium, evaluated under laboratory and greenhouse conditions.

Treatment ¹	Laboratory Condition			Greenhouse Condition		
	GE ^{2,3} (%)	SGE (plant/day)	MGT (day)	GE (%)	SGE (plant/day)	MGT (day)
T1	49 ^b	4.80 ^b	1.70 ^c	38 ^{b-d}	3.17 ^{b-c}	1.12 ^b
T2	50 ^b	4.83 ^b	2.09 ^{a-c}	30 ^d	2.29 ^d	1.09 ^b
T3	60 ^{ab}	5.91 ^{ab}	2.59 ^a	35 ^{cd}	2.79 ^{cd}	1.59 ^a
T4	56 ^{ab}	5.48 ^{ab}	2.66 ^a	41 ^{ab}	3.71 ^{a-c}	1.66 ^a
T5	60 ^{ab}	5.86 ^{ab}	2.54 ^a	41 ^{ab}	2.92 ^{cd}	1.54 ^a
T6	65 ^a	6.48 ^a	2.36 ^{ab}	47 ^a	4.54 ^a	1.36 ^{ab}
T7	62 ^a	5.50 ^{ab}	2.39 ^{ab}	43 ^{ab}	3.98 ^{ab}	1.39 ^{ab}
T8	48 ^b	4.71 ^b	1.96 ^{bc}	37 ^{b-d}	3.00 ^{cd}	1.66 ^a
T9	52 ^b	5.14 ^b	2.10 ^{a-c}	35 ^{cd}	3.96 ^{ab}	1.33 ^{ab}
T10	52 ^b	5.40 ^{ab}	2.57 ^a	33 ^{cd}	3.54 ^{b-d}	1.57 ^a
F-test	*	*	**	*	*	**
C.V.%	9.21	13.28	15.12	27.13	33.43	23.25

ns, *, **: not significantly different, significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.

¹T1 = non-pelleted seeds, T2 = pelleted seeds + vermiculite, T3 = pelleted seeds + mancozeb 0.1 g.ai., T4 = pelleted seeds + mancozeb 0.2 g.ai., T5 = pelleted seeds + mancozeb 0.3 g.ai., T6 = pelleted seeds + mancozeb 0.4 g.ai., T7 = pelleted seeds + fosetyl-aluminium 0.1 g.ai., T8 = pelleted seeds + fosetyl-aluminium 0.2 g.ai., T9 = pelleted seeds + fosetyl-aluminium 0.3 g.ai., and T10 = pelleted seeds + fosetyl-aluminium 0.4 g.ai.

²Data are transformed by the arcsine before statistical analysis and back transformed data are presented. ³Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

and 6 g.ai. did not harm seed quality or seedling growth; instead, it increased germination rates (Kangsopa & Jeephet, 2021). Manganese, another component, plays a vital role in plant metabolism, including respiration and photosynthesis, improving energy utilization efficiency (Dordas, 2009). Pelleting seeds with fosetyl-aluminium at 0.1 g.ai. showed less impact on seed quality compared to higher concentrations. This indicates that fosetyl-aluminium affects marigold seed quality more than mancozeb at the same concentration. However, in terms of germination rate, fosetyl-aluminium at 0.1 g.ai. had a similar percentage to mancozeb at 0.4 g.ai. Fosetyl-aluminium is a systemic fungicide that quickly penetrates seeds or seedlings (Chamkasem, 2017). This mechanism may affect seed quality faster than mancozeb. Additionally, fosetyl-aluminium enhances seed germination by boosting the plant's immune system, leading to faster germination.

Under laboratory conditions, changes in seedling growth indicated that seed pelleting with mancozeb at 0.4 g.ai., fosetyl-aluminium at 0.2 g.ai., and fosetyl-aluminium at 0.4 g.ai. resulted in greater shoot lengths compared to other treatments. Seed pelleting with mancozeb at 0.2 g.ai. and fosetyl-aluminium at 0.1 g.ai. showed slightly higher root lengths than other treatments. However, non-pelleted seeds and seeds pelleted with fosetyl-aluminium at 0.2 g.ai. exhibited lower root fresh weights compared to other treatments. Under greenhouse conditions, seed pelleting with mancozeb at 0.4 g.ai. and all four concentrations of fosetyl-aluminium resulted in significantly greater shoot lengths compared to non-pelleted seeds. Additionally, seeds pelleted with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. had higher shoot fresh weights than other treatments. Considering the effects on marigold seedling growth after seed pelleting with mancozeb and fosetyl-aluminium at four different concentrations, the results indicated that marigold seedlings generally exhibited improved quality. However, pelleting with fosetyl-aluminium at concentrations exceeding 0.1 g.ai. negatively affected both shoot and root elongation under laboratory conditions. At concentrations above 0.1 g.ai., fosetyl-aluminium negatively affected both shoot and root elongation under laboratory conditions (Table 4). This inhibitory effect may result from excessive phosphate uptake, which causes cellular imbalance and disrupts normal cell division and elongation. Fosetyl-aluminium functions by competing with or displacing phosphates in allosteric enzymes, thereby impairing ATP synthesis and nucleic acid replication (Ibrahim et al., 2022; Haq et al., 2023). Since phosphates are essential for energy production, cellular signaling, protein synthesis, and cell division, their disruption ultimately compromises early seedling development. In contrast, mancozeb did not show such adverse effects, as it contains zinc that supports auxin synthesis and promotes normal root and shoot growth (Stanković et al., 2010).

3.3 Effects of fungicidal seed pelleting on germination and storage longevity

After selecting two effective fungicides, mancozeb and fosetyl-aluminium, from an initial group of six, further tests were conducted to evaluate their impact on seed quality. The tests identified two formulations, mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai., that did not adversely affect seed quality and were effective in inhibiting *Pythium* sp. These formulations were then stored under various conditions for six months to assess their impact on seed quality and their efficacy in inhibiting *Pythium* sp. Under controlled conditions, laboratory tests revealed that two months after pelleting, the germination percentage of all treated seeds was similar to that of non-pelleted seeds. However, at four

Table 4. Shoot length (SHL), root length (RHL), shoot fresh weight (SFW), shoot dry weight (SDW) and root fresh weight (RFW) parameters of marigold seedlings from seeds pelleted with different concentrations of mancozeb and fosetyl-aluminium under laboratory and greenhouse conditions

Treatment ¹	Laboratory Condition ²				Greenhouse Condition ²	
	SHL (g)	RHL (g)	SFW (mg)	RFW (mg)	SHL (g)	SFW (mg)
T1	5.26 ^{ab}	3.42 ^{ab}	330 ^{ab}	410 ^b	3.89 ^b	408 ^b
T2	5.03 ^b	3.35 ^{ab}	320 ^{ab}	430 ^{ab}	4.14 ^{ab}	400 ^b
T3	5.66 ^{ab}	3.25 ^{ab}	350 ^a	440 ^{ab}	3.10 ^{bc}	350 ^{bc}
T4	6.03 ^{ab}	3.84 ^a	340 ^a	490 ^{ab}	2.94 ^c	300 ^c
T5	5.22 ^{ab}	3.57 ^{ab}	340 ^a	470 ^{ab}	2.91 ^c	380 ^{bc}
T6	6.56 ^a	3.59 ^{ab}	350 ^a	540 ^a	4.44 ^a	590 ^a
T7	5.76 ^{ab}	3.89 ^a	340 ^a	532 ^a	4.59 ^a	570 ^a
T8	6.73 ^a	3.12 ^b	250 ^b	411 ^b	4.43 ^a	430 ^{ab}
T9	5.81 ^{ab}	3.10 ^b	290 ^b	420 ^{ab}	4.35 ^a	420 ^{ab}
T10	6.62 ^a	3.55 ^{ab}	320 ^{ab}	460 ^{ab}	4.41 ^a	480 ^{ab}
F-test	**	*	**	**	*	**
C.V.%	13.62	15.79	8.46	20.38	20.62	11.03

*, **: significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.

¹T1 = non-pelleted seeds, T2 = pelleted seeds + vermiculite, T3 = pelleted seeds + Mancozeb 0.1 g.ai., T4 = pelleted seeds + Mancozeb 0.2 g.ai., T5 = pelleted seeds + Mancozeb 0.3 g.ai., T6 = pelleted seeds + Mancozeb 0.4 g.ai., T7 = pelleted seeds + Fosetyl-aluminium 0.1 g.ai., T8 = pelleted seeds + Fosetyl-aluminium 0.2 g.ai., T9 = pelleted seeds + Fosetyl-aluminium 0.3 g.ai., and T10 = pelleted seeds + Fosetyl-aluminium 0.4 g.ai. ²Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

and six months, seeds pelleted with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. showed significantly higher germination percentages ($p \leq 0.05$, DMRT) compared to non-pelleted seeds. In greenhouse conditions, seeds pelleted with mancozeb at 0.4 g.ai. exhibited the highest germination rates immediately after pelleting. After two, four, and six months of storage, seeds treated with mancozeb at 0.4 g.ai. maintained significantly higher germination percentages compared to other treatments (Table 5). Under ambient conditions, laboratory tests showed no significant differences in germination percentages among the treatments immediately after pelleting. However, after two and four months of storage, seeds pelleted with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. had higher germination percentages than other treatments. After six months, seeds pelleted with fosetyl-aluminium at 0.1 g.ai. had germination percentages similar to non-pelleted seeds. In greenhouse conditions, both immediately after pelleting and after four months of storage, seeds treated with mancozeb at 0.4 g.ai. showed significantly higher germination percentages compared to non-pelleted seeds. After six months, the germination percentages of all treatments, including non-pelleted seeds, decreased rapidly (Table 5). This decline may be attributed to prolonged exposure to storage conditions, which can lead to seed deterioration, reduced viability, and depletion of seed reserves essential for germination.

Table 5. Germination percentage of marigold seeds pelleted with different fungicide treatments after 6 months of storage.

Treatment ¹	Germination (%) -Laboratory ^{2,3}				Germination (%) -Greenhouse			
	After pelleted	2 months	4 months	6 months	After pelleted	2 months	4 months	6 months
Controlled condition								
T1	57	52	39 ^b	31 ^b	44 ^b	37 ^{bc}	30 ^c	26 ^b
T2	58	51	41 ^a	49 ^{ab}	54 ^{ab}	33 ^c	28 ^c	25 ^b
T3	60	51	41 ^a	42 ^a	60 ^a	53 ^a	44 ^a	33 ^a
T4	61	52	42 ^a	41 ^a	56 ^{ab}	40 ^b	39 ^b	26 ^b
F-test	ns	ns	*	*	*	**	*	*
C.V.%	5.41	6.11	12.45	5.48	12.68	11.51	16.47	15.17
Ambient condition								
T1	57	49 ^b	31 ^b	22 ^b	44 ^b	33 ^{ab}	34 ^b	17 ^b
T2	58	48 ^b	44 ^a	25 ^{ab}	54 ^{ab}	28 ^b	37 ^b	20 ^{ab}
T3	60	60 ^a	42 ^a	29 ^a	60 ^a	49 ^a	45 ^a	21 ^{ab}
T4	61	58 ^a	45 ^a	23 ^b	56 ^{ab}	35 ^{ab}	42 ^{ab}	23 ^a
F-test	ns	*	*	*	*	*	*	*
C.V.%	5.41	8.12	14.54	5.68	12.68	11.08	16.47	19.79

ns,*,**: not significantly different, significantly difference at $P \leq 0.05$ and $P \leq 0.01$, respectively

¹T1 = non-pelleted seeds, T2 = pelleted seeds + vermiculite, T3 = pelleted seeds + Mancozeb 0.4 g.ai., T4 = pelleted seeds + Fosetyl-aluminium 0.1 g.ai. ²Data transformed by the arcsine before statistical analysis and back transformed data are presented. ³Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

Considering the seed germination quality after six months of storage under both conditions, it is evident that seed quality declined significantly after four months of storage. However, the test results indicate that pelleted seeds maintain higher germination quality compared to non-pelleted seeds. This is because pelleted seeds are pelleted with filler material and binding agents, protecting them from unfavorable external environmental factors (Siri, 2015). Additionally, the pelleting material enhances moisture absorption and provides protection from adverse conditions through the action of fungicides such as mancozeb and fosetyl-aluminium. The results also show that seeds pelleted with mancozeb at 0.4 g.ai. had significantly higher germination percentages than non-pelleted seeds. This was likely due to the presence of zinc and manganese in mancozeb, which promoted protein and enzyme synthesis critical for cell division during germination. Moreover, zinc stimulates the production of auxins, which aid in cell elongation and the growth of roots and shoots (Stanković et al., 2010). Therefore, pelleting seeds with mancozeb at 0.4 g.ai. effectively enhanced germination rates up to four months of storage under both conditions.

3.4 Effects of fungicidal seed pelleting on *Pythium* sp. suppression and seedling viability

Under controlled conditions, laboratory tests revealed that after pelleting and testing for inhibition of *Pythium* sp., seed pelleting with mancozeb at 0.4 g.ai. achieved 100% inhibition, while pelleting with fosetyl-aluminium at 0.1 g.ai. resulted in 89% inhibition. In contrast, the non-pelleted seeds showed only 12% inhibition (Table 6). After two months of storage, the inhibition percentages for seeds pelleted with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai., a sharp decline in inhibition efficacy was observed after the first two months, followed by a slower reduction over the subsequent months ranging from 24% to 35%. Tests conducted under ambient conditions showed similar results, with inhibition percentages decreasing after two months of storage. Viability tests of seedlings after storing seeds under controlled conditions and testing in a greenhouse showed that seeds pelleted with mancozeb at 0.4 g.ai. and fosetyl-aluminium at 0.1 g.ai. maintained high *Pythium* sp. inhibition percentages of 63% and 65%, respectively (Table 6). However, after two and four months of storage, the viability of seedlings from seeds pelleted with these fungicides decreased, with average percentages ranging from 38% to 47%. After six months of storage, the viability percentages further decreased by about 10%. Similarly, seeds stored under ambient conditions showed that seeds pelleted with mancozeb at 0.4 g.ai. maintained seedling viability at rates 30-40% higher than non-pelleted seeds after six months of storage (Table 6). The results of seed pelleting with fungicides indicated that immediately after pelleting, the treated seeds maintained high inhibition percentages against *Pythium* sp., particularly with mancozeb at 0.4 g.ai. However, laboratory tests showed a significant reduction in fungicidal efficacy, by approximately 60-70%, after two months of storage. When tested in greenhouse conditions, the viability of seedlings increased by 53-56%. Mancozeb, a contact fungicide, may degrade more rapidly upon exposure to the environment, whereas fosetyl-aluminium, a systemic fungicide, tends to persist longer due to its mode of action within plant tissues (Silva et al., 2016; Dall'Agnol et al., 2021). Despite storage, seeds pelleted with fungicides maintained higher seedling viability percentages compared to non-pelleted seeds. The decline in fungicidal efficacy could be attributed to various factors affecting the seeds after pelleting and during storage, such as light, moisture, and temperature. Light, especially ultraviolet (UV) radiation from sunlight, can induce photodegradation, altering the chemical structure of fungicides and reducing their efficacy (Papadakis et al., 2018; Marsala et al., 2020; Gikas et al., 2022). Moisture can cause hydrolysis, leading to the decomposition and degradation of fungicides. Temperature can accelerate thermal decomposition, causing fungicides to evaporate or degrade more rapidly (Gikas et al., 2022). These factors may reduce the efficacy of the active ingredients on the seeds during the storage period.

Table 6. Inhibition of *Pythium* sp. and seedling viability of marigold seeds pelleted with fungicides after 6 months of storage under laboratory and greenhouse conditions

Treatment ¹	Laboratory				Greenhouse			
	Inhibition ^{2,3} (%)				Viability of Seedlings ^{2,3} (%)			
	After pelleted	2 months	4 months	6 months	After pelleted	2 months	4 months	6 months
Controlled condition								
T1	0 ^c	0 ^c	0 ^c	0 ^b	10 ^c	6 ^c	3 ^c	2 ^c
T2	12 ^b	10 ^b	5 ^b	0 ^b	23 ^b	27 ^b	20 ^b	13 ^b
T3	100 ^a	35 ^a	30 ^a	24 ^a	63 ^a	47 ^a	41 ^a	30 ^a
T4	89 ^{ab}	32 ^a	28 ^a	26 ^a	65 ^a	40 ^a	38 ^a	28 ^a
F-test	**	**	**	**	**	**	**	**
C.V.%	23.09	9.03	10.11	21.23	12.08	9.88	12.67	9.07
Ambient condition								
T1	0 ^c	0 ^c	0 ^c	0 ^c	10 ^c	4 ^c	1 ^c	0 ^c
T2	12 ^b	12 ^b	2 ^{bc}	0 ^c	23 ^b	25 ^b	16 ^b	15 ^b
T3	100 ^{ab}	31 ^a	28 ^a	19 ^a	63 ^a	45 ^a	39 ^a	29 ^a
T4	89 ^{ab}	30 ^a	26 ^a	22 ^a	65 ^a	40 ^a	32 ^a	27 ^a
F-test	**	**	**	**	**	**	**	**
C.V.%	23.09	12.30	14.31	12.02	12.08	9.08	11.31	18.07

** : significantly different at $P \leq 0.01$.

¹ T1 = non-pelleted seeds, T2 = pelleted seeds + vermiculite, T3 = pelleted seeds + Mancozeb 0.4 g.ai., T4 = pelleted seeds + Fosetyl-aluminium 0.1 g.ai. ² Data transformed by the arcsine before statistical analysis and back transformed data are presented. ³ Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

4. Conclusions

The ability of various types and concentrations of fungicides to prevent diseases caused by *Pythium* sp. in marigold was tested via pelleting of the marigold seeds was assessed in this study. Among the fungicides tested, mancozeb at 0.4 g.ai. was the most effective for the suppression of *Pythium* sp. in the marigold seeds. This treatment significantly enhanced germination, seedling vigor, and storage viability for up to four months. Fosetyl-aluminium at 0.1 g.ai. also improved seed quality and suppressed disease; however, its efficacy declined gradually over time and remained slightly lower than that of mancozeb. This reduced persistence may be attributed to the systemic nature of fosetyl-aluminium, which is more susceptible to chemical degradation and moisture-induced breakdown during storage, compared with the multi-site contact action and higher stability of mancozeb. These results demonstrate that fungicidal seed pelleting with either mancozeb or fosetyl-aluminium is a practical strategy for improving marigold seed health. Future studies should evaluate their performance against other economically important seedborne pathogens and assess long-term efficacy under field conditions.

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6. Authors' Contributions

Aranya Singsopa designed and conducted the experiments, performed data collection and statistical analysis, and prepared the initial draft of the manuscript. Nararat Thawong and Chatsuda Phuakjaiphaeo assisted in laboratory preparation, seed pelleting, pathogen inoculation, and greenhouse evaluations. Sutheera Hermhuk contributed to data interpretation, figure preparation, and critical revision of the manuscript. Jakkrapong Kangsopa conceptualized the research framework, supervised all experimental stages, provided scientific guidance, and finalized the manuscript as the corresponding author. All authors discussed the results, reviewed, and approved the final version of the manuscript for submission.

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7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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